

Letter to the Editor

 α -Purothionin, a new wheat allergen associated with severe allergy

To the Editor:

Since the first reports of IgE-mediated sensitization to wheat-containing food,¹ wheat (*Triticum aestivum*) has been recognized as an important source for food allergens in children and adults, causing organ-specific manifestations in the gut, skin, and respiratory tract, as well as severe systemic anaphylactic reactions.²

Here we report the molecular and immunologic characterization of a new wheat food allergen, α -purothionin. Five IgE-reactive cDNA clones (ie, clones 7, 12, 24, 30, and 84; Fig 1) coding for C-terminal portions of α -purothionin (<http://www.ncbi.nlm.nih.gov/genbank/gi4007850>) were isolated from a wheat seed cDNA expression library³ by using serum IgE from a patient who had experienced anaphylactic reactions on wheat ingestion (patient 1, see Table E1 in this article's Online Repository at www.jacionline.org), suggesting that the C-terminus of the allergen contains the major IgE epitopes. The allergen was designated by the World Health Organization–International Union of Immunological Societies allergen standardization subcommittee as Tri a 37.

The sequence identity of α -purothionins from wheat, rye, and barley was high (ie, >80%), whereas α -purothionins from oat, rice, and other plants showed a much lower sequence identity of 50% or less (Fig 1).

The calculated molecular weight of recombinant wheat α -purothionin without leader peptide and an N-terminal methionine is 12.7 kDa, and the calculated isoelectric point is 6.27. Tri a 37 contains a hydrophobic signal peptide, an approximately 5 kDa N-terminal thionin domain, and a C-terminal acidic extension domain. α -Purothionin is a member of a new allergen family belonging to the pathogenesis-related protein 13 family, which has been shown to lyse membranes of attacking pathogens, presumably by binding with its thionin domain to phospholipids in the membranes of the pathogens.^{4,5}

rTri a 37 was expressed in *Escherichia coli* as a His-tagged protein and purified to homogeneity. When we tested rTri a 37 for IgE reactivity with sera from patients with wheat food allergy (n = 103) from different European countries in IgE dot blot experiments, we obtained comparable percentages of positive sera in the different populations (ie, Finland, 11%; Austria and Italy, 20%; and Greece, 23%). Only 3% (2/59) of patients with baker's asthma showed IgE reactivity to rTri a 37 (data not shown). Thus

IgE reactivity to rTri a 37 was highly specific for wheat food allergy because patients with grass pollen allergy and nonallergic subjects did not show IgE reactivity (Fig 2). In the tested population of patients with wheat food allergy (n = 103), rTri a 37 was specifically recognized by approximately 16% of the patients with different intensities (Fig 2), whereas among the ones that could be tested for IgE reactivity to omega-5-gliadin (n = 67),⁶ 39% were positive.

A grading of symptoms and definition of anaphylaxis was performed according to an international position paper and recommendations for the definition of anaphylaxis in a blinded manner (see Table E1).⁷ Twenty-nine (28%) of 103 patients with wheat food allergy had wheat-induced anaphylaxis. Nine (56%) of the 16 patients with positive results for rTri a 37 and 20 (23%) of the 87 patients with wheat allergy without rTri a 37-specific IgE had wheat-induced anaphylaxis ($P = .013$). Estimate of the relative risk by means of logistic regression indicated that the risk of wheat-induced anaphylaxis is more than 4-fold higher in the rTri a 37-reactive patients (odds ratio, 4.6; 95% CI, 1.5–14.2). Although Tri a 37 is not a major wheat allergen, it might be considered a potential diagnostic marker for severe anaphylactic reactions to wheat in sensitized patients. No correlation between age and rTri a 37 reactivity or anaphylaxis was found (data not shown). Among 26 patients with positive results for omega-5-gliadin, 13 (50%) had anaphylaxis, whereas among patients with negative results for omega-5-gliadin, this percentage was significantly ($P = .018$) lower (22%). The odds ratio was 3.6 (95% CI, 1.2–10.7). There was a moderate correlation between rTri a 37 and omega-5-gliadin (contingency coefficient, 0.3; $P = .01$). Two of the sera that were negative with omega-5-gliadin were positive with rTri a 37 (patients 1 and 11, see Table E1).

Wheat allergen-specific IgE levels were not always related with wheat-induced anaphylaxis. For example, patients 11 and 12 experienced wheat-induced anaphylaxis but exhibited low wheat allergen-specific IgE levels (<12 kilounits of antigen per liter).

When compared with wheat extract, Tri a 37 was able to induce basophil degranulation, indicating its allergenic activity (see Table E1).⁸ Although the ability of Tri a 37 and wheat extract to induce mediator release was lower than that observed with respiratory allergens, it is possible that sensitized patients experience strong anaphylactic reactions when large amounts of wheat are ingested.⁹

Using Tri a 37-specific rabbit antibodies, we found that the level of Tri a 37 expression was highest in mature wheat seeds

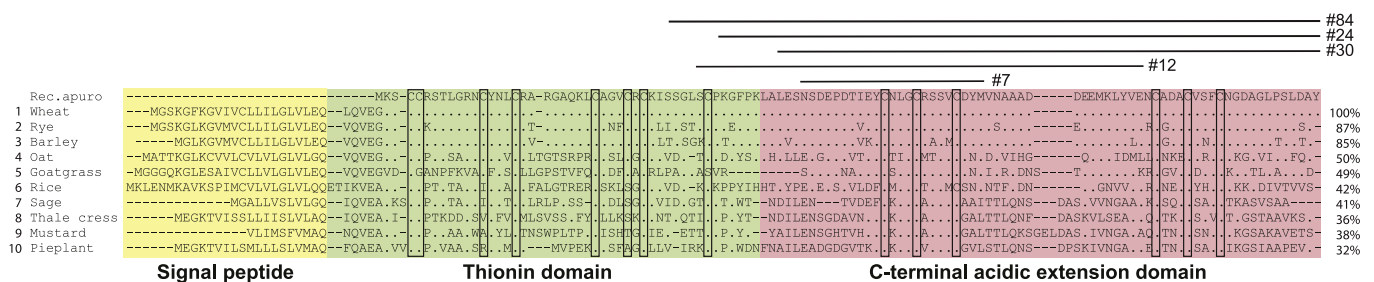


FIG 1. Sequence identities (percentages) of wheat α -purothionin with homologous proteins in other plants. Conserved cysteine residues are boxed; dashes represent gaps, and dots represent identical amino acids.

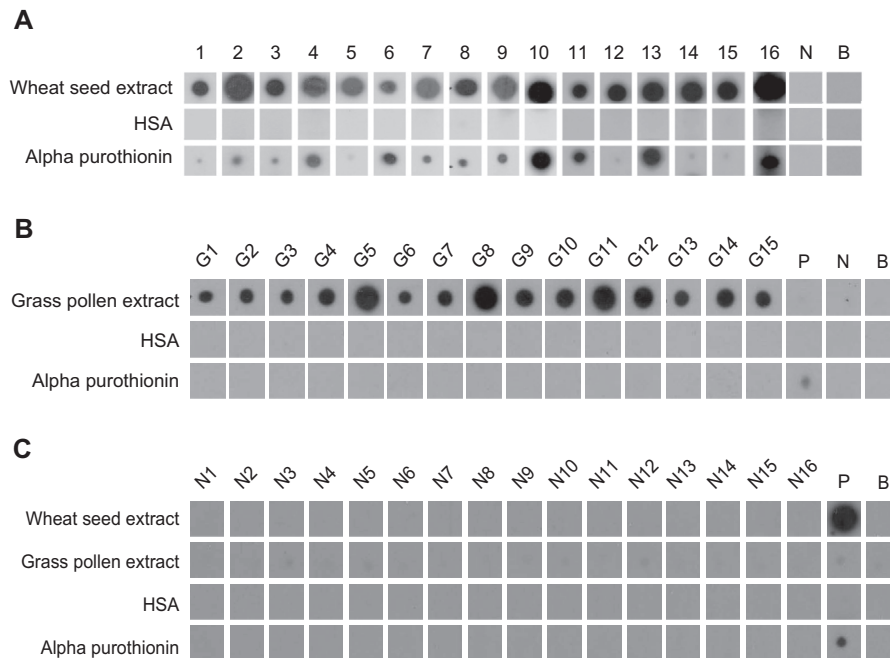


FIG 2. IgE reactivity of patients with wheat food allergy (patients 1-16; **A**), patients with grass pollen allergy (patients G1-G15; **B**), or nonallergic subjects (patients N1-N16; **C**) to rTri a 37 (α -purothionin). For control purposes, serum from a nonallergic subject (N), buffer alone (B), or serum from a patient with α -purothionin allergy (P; patient 2 in Table E1) were included. HSA, Human serum albumin.

(see Fig E1, B, in this article's Online Repository at www.jacionline.org; 15-18 kDa and higher molecular weight bands, possibly dimers and trimers at approximately 35 and 50 kDa). In mature wheat seeds (cultivar Grandios), natural α -purothionin becomes detectable at 15 kDa, with additional bands at 25 and 35 kDa possibly representing dimers and trimers. Immunologic cross-reactivity was only found for α -purothionins from rye (monomeric form at 15 kDa) and barley (monomeric form at 14 kDa, see Fig E2 in this article's Online Repository at www.jacionline.org) but not from maize, oat, rice, rye, soy, spelt, and sunflower (data not shown). Additionally, the allergen could be detected in processed wheat products, such as rolls and breads (20-50 kDa, see Fig E3 in this article's Online Repository at www.jacionline.org), indicating that it represents a highly stable protein that can resist baking. Because Tri a 37 could not be detected in gluten-free bread, the latter might be considered as an alternative diet for Tri a 37-monosensitized patients, for whom sensitization to other wheat allergens has been excluded.

In conclusion, Tri a 37 is a novel water- and salt-soluble wheat food allergen that can be found in the albumin/globulin fraction. It belongs to a new family of pathogenesis-related proteins not yet described to be involved in plant food allergy. Similar to certain other food allergens used as markers for anaphylaxis (eg, Cor a 8),¹⁰ rTri a 37 was frequently recognized by patients with severe anaphylactic reactions to wheat. Therefore in sensitized patients Tri a 37 might represent a diagnostic marker for an increased risk of wheat-induced anaphylaxis.

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REFERENCES

- Goldstein GB, Heiner DC, Rose B. Studies of reagins to alpha-gliadin in a patient with wheat hypersensitivity. *J Allergy* 1969;44:37-50.
- Helm RM, Burks AW. Food allergens. *Clin Allergy Immunol* 2008;21:219-35.

3. Constantin C, Quirce S, Grote M, Touraev A, Swoboda I, Stoecklinger A, et al. Molecular and immunological characterization of a wheat serine proteinase inhibitor as a novel allergen in baker's asthma. *J Immunol* 2008;180:7451-60.
4. Garcia-Olmedo F, Molina A, Alamillo JM, Rodriguez-Palenzuela P. Plant defense peptides. *Biopolymers* 1998;47:479-91.
5. Stec B, Markman O, Rao U, Heffron G, Henderson S, Vernon LP, et al. Proposal for molecular mechanism of thionins deduced from physico-chemical studies of plant toxins. *J Pept Res* 2004;64:210-24.
6. Palosuo K, Varjonen E, Kekki OM, Klemola T, Kalkkinen N, Alenius H, et al. Wheat omega-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. *J Allergy Clin Immunol* 2001;108:634-8.
7. Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report—second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *Ann Emerg Med* 2006;47:373-80.
8. Vogel L, Luttkopf D, Hatahet L, Haustein D, Vieths S. Development of a functional in vitro assay as a novel tool for the standardization of allergen extracts in the human system. *Allergy* 2005;60:1021-8.
9. Pahr S, Constantin C, Mari A, Scheiblhofer S, Thalhamer J, Ebner C, et al. Molecular characterization of wheat allergens specifically by patients suffering from wheat-induced respiratory allergy. *Clin Exp Allergy* 2012;42:597-609.
10. Schocker F, Luttkopf D, Scheurer S, Petersen A, Cistero-Bahima A, Enrique E, et al. Recombinant lipid transfer protein Cor a 8 from hazelnut: a new tool for in vitro diagnosis of potentially severe hazelnut allergy. *J Allergy Clin Immunol* 2004;113:141-7.

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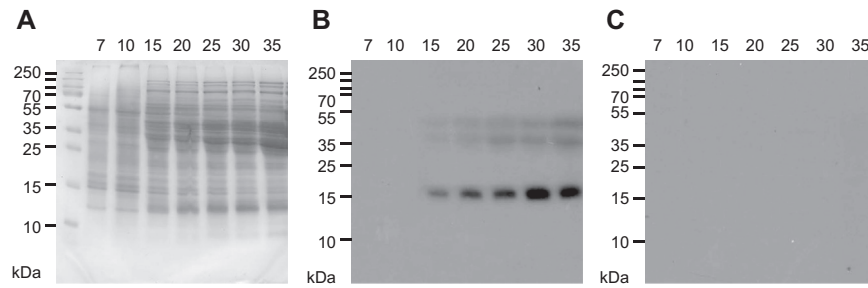


FIG E1. Accumulation of Tri a 37 in wheat seeds during maturation. Wheat extracts from wheat seeds at different times of maturation (days 7, 10, 15, 20, 25, 30, and 35) were separated by means of SDS-PAGE and stained with Coomassie blue (**A**), blotted onto nitrocellulose, and tested with α -purothionin-specific rabbit antibodies (**B**) or the corresponding preimmune serum (**C**).

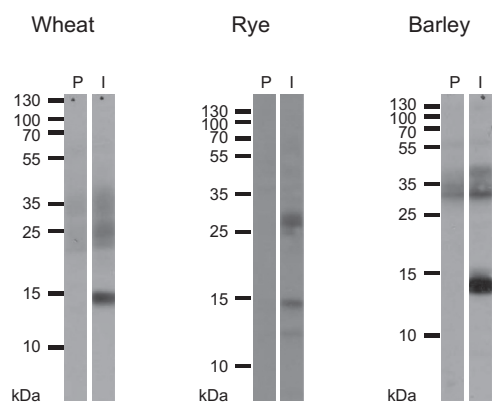


FIG E2. Detection of α -purothionin-related proteins in rye and barley. Nitrocellulose-blotted wheat, rye, and barley seed extracts were tested with α -purothionin-specific rabbit antibodies (*lanes I*) or with the corresponding preimmune serum (*lanes P*). Molecular weights are indicated at left in kilodaltons.

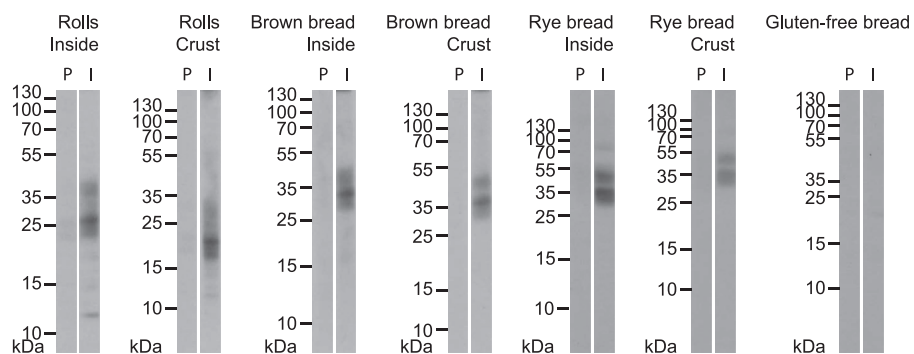


FIG E3. Detection of α -purothionin in different types of bread. Nitrocellulose-blotted extracts from rolls, brown bread, and rye bread (inside; crust), as well as gluten-free bread, were incubated with α -purothionin-specific rabbit antibodies (*lanes I*) or the corresponding preimmune serum (*lanes P*). Molecular weights are indicated at left in kilodaltons.

TABLE E1. Demographic, clinical, and serologic characterization of patients with wheat food allergy with IgE reactivity to α -purothionin

Patient no.	Age (y)	Sex	Wheat-induced symptoms	Wheat-induced anaphylaxis	Total IgE (kU/L)	Wheat-specific IgE (kU _A /L)	Wheat challenge	Wheat SPT, mean wheal diameter (mm)	IgE sensitizations to other food	Other IgE sensitizations	Release (%) WSE (1000 ng/mL)	Release (%) rTri a 37 (100 ng/mL)	Reactions after
1	12	F	AS, GIS, SS	Yes	562	68.8	Not done	4	No	No	19.14	5.44	Wheat-containing products
2	41	F	AS, GIS	Yes	252	180	Not done	Positive	r	c, hdm	15.13	7	Wheat-containing products
3	19	M	AS, GIS, SS	Yes	653	339	Not done	Positive	e, n, r	b, gp	21.11	5.63	Wheat-containing products
4	9	M	IM	No	5031	>100	Open (positive)	5	ba, e, o, r	gp	18.19	8.15	Open challenge (age 6 y; symptoms: IM)
5	20	M	IM	No	437	85.7	Open (positive)	7	ba, co, e, o, r, s	b, gp	ND	ND	Open challenge (age 17 y; symptoms: IM)
6	5	F	SS	No	306	4	Open (positive)	5	ba, bu, co, e, m, o, r, s	No	11.42	3.74	Open challenge (age 2 y; symptoms: SS)
7	6	F	SS	No	1429	709	Open (positive)	8	e, m, r, s	No	15.18	5.39	Open challenge (age 4 y; symptoms: SS)
8	5	M	SS	No	7559	45.2	Open (positive)	6	ba, bu, co, e, m, s,	b, gp	19.58	16.13	Open challenge (age 3 y; symptoms: SS)
9	6	M	SS	No	514	>100	Open (positive)	8	ba, e, m, o, r	No	16.2	5.78	Open challenge (age 4 y; symptoms: SS)
10	14	M	AS, SS	Yes	636	>200	Open (positive)	8	e	No	ND	ND	Open challenge (age 11 y; symptoms: AS, SS)
11	3	M	GIS, SS	Yes	165	11.7	Not done	8	No	No	4.49	4.98	Pasta soup, baby cereals
12	3	M	AS, SS	Yes	349	8.09	Not done	4	e	No	5.01	3.64	Pasta
13	3	F	SS	No	795	165	Not done	7.5	e	No	19.55	6.83	Pasta
14	5	M	AS, SS	Yes	2723	695	Not done	7	No	No	20.63	4.46	Bread
15	12	F	AS, SS	Yes	4539	1491	Open (positive)	11	No	No	19.73	4.28	Open challenge (age 11 y; symptoms: severe anaphylaxis)
16	10	M	AS, GIS	Yes	802	456	Not done	7	cm	No	ND	ND	Cereals

Positive release is defined as greater than 5%.

AS, Airway symptoms; b, birch; ba, barley; bu, buckwheat; c, cat; cm, cow's milk; co, corn; e, hen's egg; F, female; GIS, gastrointestinal symptoms; gp, grass pollen; hdm, house dust mite; IM, itching of mouth; kU_A/L, kilounits of antigen per liter; M, male; m, millet; n, nut (hazelnut); ND, not done; o, oat; r, rye; s, soy; SPT, skin prick test; SS, skin symptoms; WSE, wheat seed extract.